

Exhibit I

N-acetyl Hexosamine Acid (Sialic Acid)

New NAc α 2,3 Gal β 1,4 Glc NAc

2' Sialic has the

(β 1,4 Gal NAcT

F + II (3,5,7)

New NAc α 2,3 Gal β 1,4 Glc NAc

\uparrow β 1,4 /

Gal NAc

New NAc α 2,3 Gal β 1,4 Glc NAc

\uparrow 3a

Fucose

To Prepare TEA (HPLC)

2 M (TEA) Acetic acid pH 5.5

(one liter)

550 ml H_2O

120 ml Glacial Acetic Acid (110/100 - 2M)

Trace w/ Triethylamine to 5.5

150+ ml

Bring to 1 liter - filter thru Millipore filters (designed for HPLC reagents)

FT assay

mm		F
0.5	1M Na cacodylate	3.5
0.2	2M MCH ₂	1.4
0.4	500mM L-fucose	2.8
1.0	100mM ATP	3.0
.8	¹⁴ C GDP-fucose	5.6
	H_2O & 20	35.7

Stock of
Sialyl α 2,3 Gal NAc FW=675

50mM - 29.671 mg

Cocktail

	cocktail	Exhaust	H_2O - Acc	H_2O + Acc (SLN 50mM)
FT3 DL	8.0	4.0	8	6 2
FT3 BK	8.0	4.0	8	6 2
FT7 SN	8.0	10.0	2	0 2

In Rxn 3 hrs 37°C, Partition ~~sp~~ Na₂HPO₄ Calcium count

Sulfated Acceptor

Spl of 20 μ l rxn

6.14 ng / 10 μ l

19.3 μ l = 2.3 mg

WED

14:05

Reaction counts
Appear to have
Worked

PARAMETER GROUP: 1
ID: 14-C 1 MIN

POS	CTIME	CPM1
001	00060	734.57 F13 BL + Occ
002	00060	398.40 F13 BL -
003	00060	1617.20 F13 BL +
004	00060	518.50 F13 BL -
005	00060	1105.50 F13 BL +
006	00060	368.40 F13 BL -

POS	CTIME	CPM1
011	00060	468.50
012	00060	425.40
013	00060	417.96
014	00060	517.50
015	00060	462.50
016	00060	467.50

TOTAL COUNTS
Sph

TOTAL COUNT RATE: 7621.9 CPM

From the HPLC Data
the counts in FT7 (SK)
Sample are only Fucose
This FT prep is inactive

Cells were transfected on 1/27 w/ mFT7 (1, 2ab, 3) and hFT3 (JBK 125)

Cells were FACS Analyzed on 1/30 - 4 plates each
Stained w/ Lx; SKx + H

	H	SKx	Lx
mFT7	-	+	-
hFT3	-	+	+

as would expect

Make cell 1% Triton extract partition off
Fucose Transferrase rxn SKH as acceptor

	Cocktail Extract	H ₂ O -	+	H ₂ O
FT3	10.5	7.5	2	2
FT7	10.5	7.5	2	2
Previous FT3(B)	10.5	4.0	5.5	2
Cocktail	4.0			3.5
0.5 NaCl	2.0			
0.2 2MMWCL	0.8			
0.4 L-fucose	1.6			
1.0 ATP	4.0			
0.8 60% fucose	3.2			
10.0 H ₂ O	40.4			

All runs worked fine

Use this extract to
Check out Sulfated
acceptor
and run these
on the HPLC

PARAMETER GROUP: 1
ID: 14-C 1 MIN

POS	CTIME	CPM	
001	00060	2677.65	FT3A
002	00060	7989.80	FT3 (new)
003	00060	1651.20	FT7
004	00060	745.80	- dm
005	00060	1945.80	- dm
006	00060	679.70	- dm
007	00060	454.50	
008	00060	457.50	
009	00060	474.50	
010	00060	454.50	

POS	CTIME	CPM
011	00060	409.40
012	00060	442.40

TOTAL COUNT RATE: 18383.6 CPM

Spl of 2000 of
extract 250 µl
count 10

HPLC runs

moch

Fucose

GDP fucose

SLX (Standard)

FT3 (new) rxn product

m FT7 reaction product - product migrates w/
SLX

Run FT assays w/ FT3 and SLX and Sulfated
acceptor. The sulfated acceptor will not
be removed over NaH_2PO_4 column, analysis
will be thru the HPLC separation

FT assay		50 mM		50 mM		eluent	
	celltail	Extract	SLX	Sul	H ₂ O		
FT3 -S1	13	5.0	2.0				Na ₂ CO ₃ 3.5
	Sul	13		2.0			M ₂ Cl ₂ 1.4
	S2	13	2.0				Lfuc 2.8
	-	13					ATP 7.0
							GDPfuc 5.6
					2.0		H ₂ O 53.2

HPLC Analysis

Unit

Fucose

GDP fucose

SLX

FT3/SLX

FT7 SLX

Acceptors
[final] 50 mM